

DDT: An Anomalously Resistant Molecule

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The Navy shares the concern of all socially responsible institutions for the protection of the environment. The research described here is part of a joint ONR and Naval Facilities Engineering Command effort in pollution abatement and control. This particular effort contributes to our better understanding the mechanisms of biodegradation through an examination of long-lasting materials. Of particular interest, is the popular pesticide DDT which has substantial economic, social, and health benefit, but whose use is being restricted because of its "polluting" effects. The research is expected to add to our understanding of the biodegradation of DDT and provide information which will be useful in promoting biodegradation of other specific substances.

An enormous number of organic chemicals and natural products enter marine waters. Some are discharged directly into the oceans, some are carried by river water or are bound to particulate matter transported by rivers entering the sea, and some originate from the atmosphere. Many of these substances pose no hazard to marine life because they are subject to rapid biodegradation by bacteria, fungi, or other microorganisms, the end result of the decomposition being the complete conversion of the substance to carbon dioxide, water, and other inorganic products. Owing to the enormous catabolic versatility of the marine microflora, few of the incoming chemicals endure for sufficiently long to create a long- or even a short-term hazard.

A few compounds are highly persistent, however. Inasmuch as the total destruction of complex organic molecules in nature results largely, if not solely, from the actions of microorganisms, the longevity of such substances must be attributable to the inability of these microscopic organisms to degrade them. Among the compounds, polluting agents, and natural products that are thus long-lived are plastics of many types, polychlorinated biphenyls (PCBs), a wide array of natural products characteristic of sediments, and the insecticide DDT (Figure 1). The resistance of biodegradation of many plastics is a useful trait because they do not rot, spoil, or undergo changes that might affect their intended use, yet this very resistance may be offensive because of the unsightly

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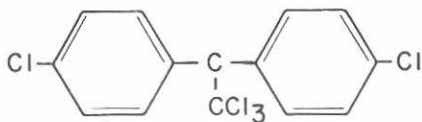


Figure 1 - Chemical formula for DDT

remains. Plastics may create other problems, such as the possible effects of small plastic particles on fish.

A few of these persistent chemicals may be hazardous to some forms of life. Evidence is accumulating, for example, that PCBs are affecting a number of different species, which fact when coupled with the ever growing reliance of society on chemicals result in an environmental problem of considerable and widespread importance. Like the PCBs, DDT is affecting a number of species, and serious question has been raised in many corners of the globe whether this effective and useful insecticide ought to be banned completely on a world-wide basis.

As a pesticide, the phenomenal value of DDT cannot be questioned. Because of its toxicity to *Anopheles* and other mosquitoes, it has saved uncounted human lives and has led to the control of malaria in many countries in the tropics. As a result, it has been said that no chemical ever found or synthesized by man has alleviated as much suffering or been as great a boon to public health as DDT. The insecticide also has been an undisputed blessing to agriculture because of its effectiveness and low cost. Yet its apparent resistance to biological degradation and its concentration through food chains (microorganisms → macroorganisms → small animals or fish → large animals or fish) have been associated with a dramatic decline in the populations of a few animal species, some of which are of economic or esthetic significance or are important species in their natural habitats.

Although the use of DDT has been terminated in the United States, the total world production remains about 150 million pounds per annum (Woodwell *et al.*, 1971). Much of the DDT that is used abroad is designed for the control of insects carrying the causative agents of communicable diseases of humans, such as malaria, or for pests that damage crops essential for countries whose populations are still under- or malnourished. A total cessation in the use of DDT in such nations, in the absence of an effective replacement that can be afforded by these poor countries, would lead to a marked increase in the incidence of human diseases transmitted by susceptible insect vectors and a decline in agricultural production in regions already lacking an adequate food supply.

Not all of the DDT sprayed onto crops and in forests remains on the plants. Some of the applied pesticide falls onto the soil and remains there probably for decades, some is transported with particles of eroding soil into rivers and streams, and a significant portion is volatilized and enters the atmosphere. The DDT, because it is not destroyed readily,

is carried to the ocean with the flow of river water or enters from atmospheric sources.

According to the estimates of Woodwell *et al.* (1971), the total amount accumulated in the marine biota is about 12 million pounds, most associated with algae and higher plants. Sedimentation of organic matter, to which DDT is bound, transports the insecticide or its metabolites to the abyss, where the insecticide may accumulate because the indigenous bacteria exhibit only limited degradative activity (Jannasch *et al.*, 1971). Furthermore, rates of transfer of organic compounds out of the abyss are of the order of hundreds to thousands of years (Woodwell *et al.*, 1971).

Given the large number of reports documenting the slow destruction of DDT in natural habitats, it seemed reasonable to conclude that DDT is not readily attacked microbiologically or that it is not even a substrate for enzymes of bacteria or fungi common in nature. Is it not perfectly plausible to believe that an extremely persistent molecule owes its longevity to the shortage of organisms containing the enzymes requisite for its breakdown or that no such organisms exist? The conclusion, however, is invalid, and numerous species capable of effecting steps in DDT metabolism have been obtained. Thus, many marine microorganisms have been found that cause some modification in the molecule, modest though the alteration is (Patil *et al.*, 1972).

The results of an experiment designed to show the frequency of aquatic bacteria able to bring about a modification of DDT are shown in Figure 2. The samples were taken from (1) brackish water from the mouth of the Connecticut River at Saybrook, Conn., (2) a salt marsh at Saybrook, (3) and (4) subtidal zone one and two miles, respectively, from the mouth of the Connecticut River in about 2 ft. of water, (5) brackish water from Oyster Creek at Saybrook, (6) subtidal zone at Kelsey Point Beach, Conn. in about 2 ft. of water (fine black sand), and (7) subtidal zone at Sagamore Terrace Beach, Conn. in about 2 ft. of water (course gravel). An alteration in the DDT in these tests is taken as the formation of water-soluble products from the DDT, a compound that is itself highly water-insoluble. The bacteria were chosen at random to obtain a reasonable approximation of their relative abundance in the test samples. The results demonstrate that from less than one-fourth to two-thirds or more of the bacteria from each sample site can convert from 5 to 10% of the DDT, provided to the cultures as the ^{14}C -labelled (radioactively tagged) compound, to water-soluble metabolites. Pfaender and Alexander (1973) found that as many as 90% of the bacteria from polluted water amended with a readily available carbon source could metabolize DDT.

Nevertheless, extensive decomposition of DDT can be brought about by the activities of two bacteria, an action that not only destroys

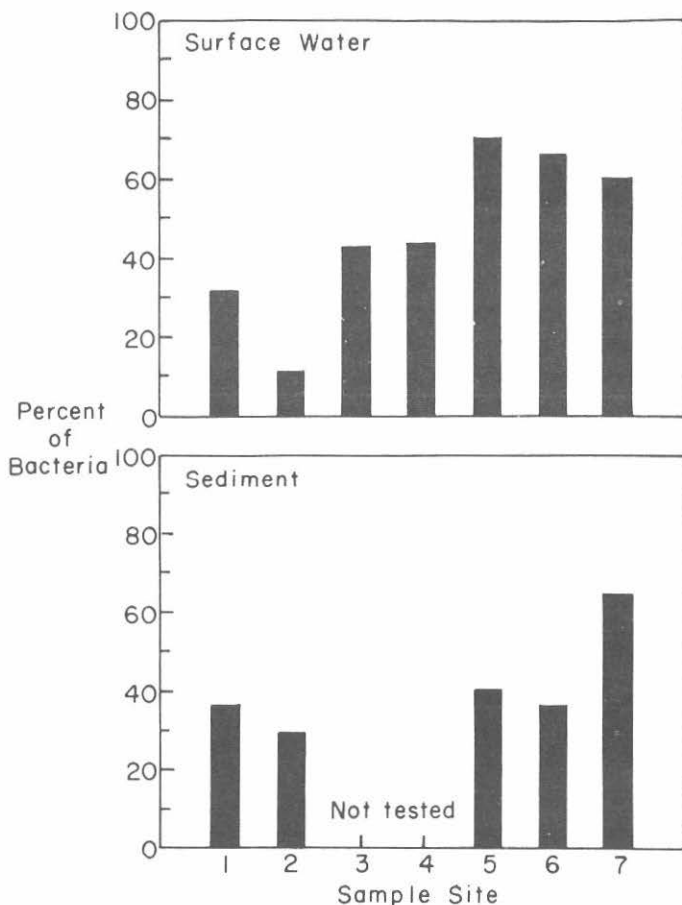


Figure 2 — Graph showing the results of an experiment designed to show the frequency of aquatic bacteria able to bring about a modification of DDT.

the molecule but one that takes place quite rapidly, at least under laboratory conditions. This process, as described by Focht and Alexander (1970a) and Pfaender and Alexander (1972), apparently involves a series of reactions in which changes are brought about in the substituents on one of the carbon atoms lying between the two benzene rings. That carbon atom is then removed from the molecule, and this removal is followed by a cleavage of one of the two benzene rings. The product is *p*-chlorophenylacetic acid ($R-CH_2-COOH$). This metabolite, in turn, is then degraded by another type of bacteria, yielding thereby a rapid and exhaustive *in vitro* degradation (Figure 3).

The behavior of DDT in natural waters is thus anomalous. On the one hand, it unquestionably fails to succumb rapidly to microbial destruction in the oceans. That is the reason that the insecticide retains its

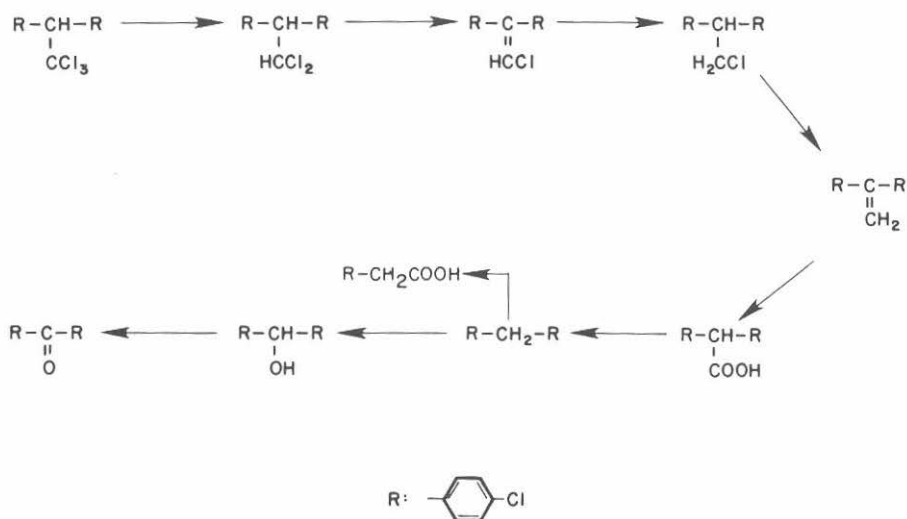


Figure 3 — Initial steps in the microbial degradation of DDT.
R is *p*-chlorophenyl.

integrity sufficiently long to appear in cells and tissues of diverse species. On the other hand, the molecule is in fact extensively metabolized by microorganisms under artificial conditions in the laboratory. What is the explanation for this anomaly? That is one of the questions whose answer is currently being sought.

A number of reasons have been proposed recently for the persistence in nature of synthetic compounds and natural products (Alexander, 1972, 1973). Some of these hypotheses have a direct bearing on the persistence of DDT. One possible explanation for its longevity is that the bacteria capable of degrading DDT are physically separated from it. Because the solubility of the insecticide in water is about 0.002 ppm and its solubility in certain lipids is about 100,000 ppm (Reinbold *et al.*, 1972), a large percentage of the DDT may be concentrated in the lipids of living organisms. DDT is known to accumulate in algae, clams, oysters, fish, and other organisms. DDT in lipids or fatty tissues and cells would be inaccessible to microorganisms. In addition, dead algae and other non-living organic material can bind DDT. Bacteria are also capable of binding DDT, as shown by Hicks and Corner (1973). They presented evidence that *Bacillus megaterium* retained about 1.7 μg of DDT/mg cells. About 75% of this DDT was found to be in the bacterial membrane, where a large portion of the cell lipid is located. It is not known whether insecticide bound to bacteria or inanimate substances is less readily available than the unbound chemical, but studies with other compounds have shown that bound, complexed, or sorbed substrates are generally not readily available for decomposition.

Another reason for the persistence of DDT may be that individual microbial populations in the ocean inhibit the species having the capacity to decompose DDT. This hypothesis is supported by a report that DDT decomposition by the fungus *Mucor alternans* is inhibited by other fungi (Anderson and Lichtenstein, 1972). The neighboring species may markedly retard the growth of organisms having the appropriate enzymes or suppress enzymatic activity. Alternatively, inasmuch as one or more of the enzymes may be inducible, conditions in nature inimical to enzyme induction, the absence of an inducer, or a concentration of inducer too low to allow for the appearance of the needed enzymes might explain why we have found that a high percentage of the isolated bacteria have the ability to convert DDT to water-soluble products *in vitro* but yet we have not found any water-soluble products in model communities of marine microorganisms.

Not only is it important to establish why DDT and some of its closely related metabolites are so persistent but it is also essential to identify the products that are generated. These products may be more persistent, more toxic, affect different species, or have different mobilities in nature than the parent molecule. The need for such studies is illustrated by the results of several investigations. For example, 2,4-dichlorophenoxyacetic acid (2,4-D) is metabolized by microorganisms in natural waters, but in the process the pesticide is converted to a product far more resistant to biodegradation and hence more persistent than the original 2,4-D (K. W. Sharpee and M. Alexander, unpublished data). A change in spectrum of toxicity as a compound is transformed is evident in a report that the pesticide thiram is converted to dimethylnitrosamine, a potential human carcinogen, in laboratory models of natural ecosystems (Ayanaba and Alexander, 1973). Thus, establishing the pathway of breakdown and characterizing the products that appear in water assume considerable significance.

It is known that DDT can be converted by bacteria to 4,4'-dichlorodiphenylmethane (Focht and Alexander, 1971; Pfaender and Alexander, 1973). If this compound is indeed produced in nature, removal of the chlorine atoms on the rings would produce diphenylmethane, which can be essentially totally degraded by a number of bacteria (Focht and Alexander, 1970b). Enzymes catalyzing the removal of a chlorine atom from organic compounds are called dehalogenases. Nonspecific dehalogenases might be involved in this conversion; however, dehalogenases appear to be highly specific and generally do not act upon even closely related molecules (Goldman, 1972).

In our search for products of DDT metabolism, the isolation of which may shed light on the pathway of degradation, it was found that *Mucor alternans* produced at least two water-soluble metabolites from DDT. Although these products have not been fully characterized, it is known

that they are not common or postulated DDT metabolites like 4,4'-dichlorodiphenylacetic acid, *p*-chlorophenylacetic acid, or 2-chlorosuccinic acid. It is likely that these water-soluble metabolites, which seem to be organic acids, are probably previously uncharacterized breakdown products. The identities of the compounds are currently being sought.

The presence of a chlorine atom on each of the two benzene rings of DDT may present a major obstacle to functioning of the enzymes which commonly cleave aromatic rings. Dagley (1971) presented evidence that *ortho*-cleaving enzymes (enzymes rupturing the benzene ring between two adjacent hydroxyl groups) are not generally active on rings with substituents, such as chlorine, *etc.*, on them. A typical *ortho*-cleavage sequence would likely produce 2-chlorosuccinic acid or some related compound (Figure 4). The presence of this compound in culture fluids of *M. alternans* was sought, but none was found. The enzymes involved in *meta*-cleavage are generally more active than *ortho*-cleaving enzymes on rings with substituents on them. A *meta*-type of ring cleavage would probably yield a 3-chloro-substituted acid from DDT (Figure 4), as suggested by Focht (1972). The formation of 3-chloro-substituted acids from DDT seems to be plausible, and a search for them in cultures of *M. alternans* and of marine bacteria will be made.

Therefore, despite the long continued applications of DDT, little is known about why it persists and how it is destroyed in marine ecosystems. Furthermore, although DDT use has been terminated or will

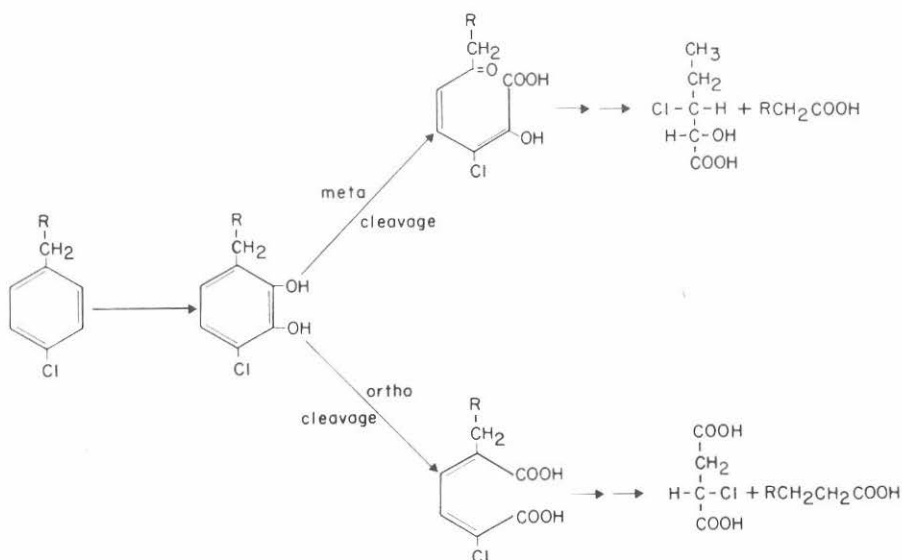


Figure 4 — Postulated pathway for the cleavage of the first benzene ring of DDT.
R is *p*-chlorophenyl.

soon be markedly curtailed in the technologically advanced countries, it will endure for many years, and additional amounts of the insecticide will probably be introduced into the oceans owing to its usefulness in public health activities and agricultural operations in the tropics. For these reasons and because the possible hazards of products of DDT transformation in nature only have been partially established, further research is needed to define more adequately the fate and pathway of breakdown of this global and persistent pollutant. In addition, since it is now evident that DDT is in fact biodegradable and not almost wholly resistant to microbial attack, as heretofore believed, it is important to account for its anomalous longevity in nature so that possible means for its destruction or alternative but nonpolluting insecticides may be found.

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